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(57) Abstract

The present invention relates to amino acid sequence variants of human DNase I that have increased DNA-hydrolytic activity. The invention provides nucleic acid sequences encoding such hyperactive variants, thereby enabling the production of these variants in quantities sufficient for clinical use. The invention also relates to pharmaceutical compositions and therapeutic uses of hyperactive variants of human DNase I.

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HUMAN DNASE I HYPERACTIVE VARIANTS

Field of the Invention

The present invention is related to results obtained from research on human deoxyribonuclease I (DNase I), a phosphodiesterase that is capable of hydrolyzing polydeoxyribonucleic acid. It relates generally to modified (variant) forms of human DNase I having increased DNA-hydrolytic activity and their preparation by recombinant DNA methods, to pharmaceutical compositions by which their utility can be exploited clinically, and to methods of using these DNase I variants and compositions thereof.

Background of the Invention

DNase I is a phosphodiesterase capable of hydrolyzing polydeoxyribonucleic acid. DNase I has been purified from various species to various degrees.

Bovine DNase I has been extensively studied biochemically. See e.g., Moore, in The Enzymes (Boyer, P.D., ed), pp. 281-296, Academic press, New York (1981). The complete amino acid sequence for bovine DNase I is known (Liao, et al., J. Biol. Chem. 248:1489-1495 (1973); Oefner, et al., J. Mol. Biol. 192:605-632 (1986); Lahm, et al., J. Mol. Biol. 221:645-667 (1991)), and DNA encoding bovine DNase I has been cloned and expressed (Worrall, et al., J. Biol. Chem 265:21889-21895 (1990)). The structure of bovine DNase I has been determined by X-ray crystallography. Suck, et al., EMBO J. 3:2423-2430 (1984); Suck, et al., Nature 321:620-625 (1986); Oefner, et al., J. Mol. Biol. 192:605-632 (1986); Weston, et al., J. Mol. Biol. 226:1237-1256 (1992).

DNA encoding human DNase I has been isolated and sequenced and that DNA has been expressed in recombinant host cells, thereby enabling the production of human DNase I in commercially useful quantities. Shak, et al., Proc. Nat. Acad. Sci. 87:9188-9192 (1990).

DNase I has a number of known utilities and has been used for therapeutic purposes. Its principal therapeutic use has been to reduce the viscoelasticity of pulmonary secretions (mucus) in such diseases as pneumonia and cystic fibrosis (CF), thereby aiding in the clearing of respiratory airways. See e.g., Lourenco, et al., Arch. Intern. Med. 142:2299-2308 (1982); Shak, et al., Proc. Nat. Acad. Sci. 87:9188-9192 (1990); Hubbard, et al., New Engl. J. Med. 326:812-815 (1992); Fuchs, et al., New Engl. J. Med. 331:637-642 (1994); Bryson, et al., Drugs 48:894-906 (1994). Mucus also contributes to the morbidity of chronic bronchitis, asthmatic bronchitis, bronchiectasis, emphysema, acute and chronic sinusitis, and even the common cold.

The pulmonary secretions of persons having such diseases are complex materials, that include mucus glycoproteins, mucopolysaccharides, proteases, actin, and DNA. Some of the materials in pulmonary secretions are released from leukocytes (neutrophils) that infiltrate pulmonary tissue in response to the presence of microbes (e.g., strains of Pseudomonas, Pneumococcus, or Staphylococcus bacteria) or other irritants (e.g.,

tobacco smoke, pollen). In the course of reacting with such microbes or irritants, the leukocytes may degenerate and release their contents, which contribute to the viscoelasticity of the pulmonary secretions.

The ability of DNase I to reduce the viscoelasticity of pulmonary secretions has been ascribed to its enzymatic degradation of the large amounts of DNA released by neutrophils. Shak, et al., Proc. Nat. Acad. Sci. 87:9188-9192 (1990); Aitken, et al., J. Am. Med. Assoc. 267:1947-1951 (1992).

The present invention is based in part on research by the inventors to study the enzymatic activity of human DNase I. This research involved the design and synthesis of various human DNase I variants, and the assay of these variants to assess their ability to hydrolyze DNA in vitro. The inventors have identified for the first time a class of human DNase I variants, termed hyperactive variants, that have increased DNA-hydrolytic activity and that are less susceptible to inhibition by sodium chloride, as compared to native human DNase I.

Because of their increased DNA-hydrolytic activity, the hyperactive variants also have increased mucolytic activity and are more effective than native human DNase I in degrading (digesting) DNA generally. Because they are less susceptible to inhibition by sodium chloride, the hyperactive variants are more effective than native human DNase I under physiological saline conditions, such as occur in pulmonary secretions and other biological fluids. Accordingly, hyperactive variants of human DNase I should be especially useful in treating patients having pulmonary secretions that comprise relatively large amounts of DNA.

It is therefore an object of the present invention to provide human DNase I variants that have greater DNA-hydrolytic activity than native human DNase I.

It is another object of the invention to provide nucleic acids encoding such hyperactive variants of human DNase I, recombinant vectors comprising such nucleic acids, recombinant host cells transformed with those nucleic acids or vectors, and processes for producing the human DNase I variants by means of recombinant DNA technology. The invention includes the use of such nucleic acids and vectors for in vivo or ex vivo gene therapy.

The invention also is directed to pharmaceutical compositions comprising the hyperactive variants of human DNase I, optionally together with a pharmaceutically acceptable excipient, as well as substantially purified antibodies that are capable of binding to such hyperactive variants.

The invention also is directed to methods of use of the hyperactive variants. Included are methods for reducing the viscoelasticity or viscous consistency of DNA-containing material in a patient, and for reducing or preventing formation of DNA-containing immune complexes in a patient, comprising administering a therapeutically effective dose of a hyperactive variant of human DNase I to the patient.

The invention is particularly directed to a method of treating a patient having a disease such as cystic fibrosis, chronic bronchitis, pneumonia, bronchiectasis, emphysema, asthma, or systemic lupus erythematosus, that comprises administering a therapeutically effective amount of a hyperactive variant of human DNase I to the patient.

These and other objects of the invention will be apparent to the ordinary artisan upon consideration of the specification as a whole.

Brief Description of the Figures

Figure 1 shows the amino acid sequence of human mature DNase I (SEQ. ID. NO. 1). The numbers indicate the sequential position of amino acid residues within the sequence.

Figures 2-4 show data for the following variants:

Q9R (SEQ. ID. NO: 2), E13K (SEQ. ID. NO: 3), E13R (SEQ. ID. NO: 4), T14K (SEQ. ID. NO: 5), T14R (SEQ. ID. NO: 6), H44K (SEQ. ID. NO: 7), H44R (SEQ. ID. NO: 8), N74K (SEQ. ID. NO: 9), N74R (SEQ. ID. NO: 10), S75K (SEQ. ID. NO: 11), T205K (SEQ. ID. NO: 12), T205R (SEQ. ID. NO: 13), E13R:N74K (SEQ. ID. NO: 14), Q9R:E13R:N74K (SEQ. ID. NO: 15), E13R:N74K:T205K (SEQ. ID. NO: 16), Q9R:E13R:N74K:T205K (SEQ. ID. NO: 17).

Figure 2 shows the activity of hyperactive variants of human DNase I determined in a supercoiled DNA digestion assay and a linear DNA digestion assay, as described in Example 3.

Figure 3 shows the activity of hyperactive variants of human DNase I determined in a DNA hyperchromicity assay, as described in Example 3.

Figure 4 shows the percent activity of native human DNase I and hyperactive variants of human DNase I determined in the presence of various concentrations of sodium chloride in a linear DNA digestion assay, as described in Example 3. Percent activity values are stated relative to the activity of each DNase I (native or variant) in the absence of added sodium chloride.

Detailed Description

As used herein, the terms "human DNase I", "native human DNase I", and "wild-type DNase I" refer to the polypeptide having the amino acid sequence of human mature DNase I set forth in Figure 1.

A "variant" or "amino acid sequence variant" of human DNase I is a polypeptide that comprises an amino acid sequence different from that of native human DNase I. Generally, a variant will possess at least 80% sequence identity, preferably at least 90% sequence identity, more preferably at least 95% sequence identity, and most preferably at least 98% sequence identity with native human DNase I. Percentage sequence identity is determined, for example, by the Fitch, et al., Proc. Nat. Acad. Sci. USA 80:1382-1386 (1983), version of the algorithm described by Needleman, et al., J. Mol. Biol. 48:443-453 (1970), after aligning the sequences to provide for maximum homology.

The terms "hyperactive variant", "human DNase I hyperactive variant", and "hyperactive variant of human DNase I" refer to a variant of native human DNase I that has increased DNA-hydrolytic activity as compared to native human DNase I.

"DNA-hydrolytic activity" refers to the enzymatic activity of native human DNase I or a variant of human DNase I in hydrolyzing substrate DNA to yield 5'-phosphorylatedoligonucleotide end products. DNA-hydrolytic activity is readily determined by any of several different methods known in the art, including analytical polyacrylamide and agarose gel electrophoresis, hyperchromicity assay (Kunitz, J. Gen. Physiol. 33:349-362 (1950); Kunitz, J. Gen. Physiol. 33:363-377 (1950)), or methyl green assay (Kurnick, Arch. Biochem. 29:41-53 (1950); Sinicropi, et al., Anal. Biochem. 222:351-358 (1994)).

A human DNase I variant having "increased DNA-hydrolytic activity" is one that hydrolyzes DNA to a greater extent than native human DNase I as determined under comparable conditions. For example, if the linear DNA digestion assay described in Example 3 is used to determine DNA-hydrolytic activity, then a human DNase I variant having increased DNA-hydrolytic activity will be one having an activity greater than native human DNase I in the assay as determined under comparable conditions. In that assay, a hyperactive variant of human DNase I typically will have at least 50% greater DNA-hydrolytic activity than native human DNase, but hyperactive variants having upwards of 10-fold greater DNA-hydrolytic activity than native human DNase I also are readily produced, especially by altering multiple amino acid residues of the native human DNase I amino acid sequence (see e.g., Figure 2).

"Mucolytic activity" refers to the reduction of viscoelasticity (viscosity) of sputum or other biological material, for example as observed upon treatment of the material with native human DNase I or a hyperactive variant of human DNase I. Mucolytic activity is readily determined by any of several different methods known in the art, including sputum compaction assay (PCT Patent Publication No. WO 94/10567, published May 11, 1994), assays using a torsion pendulum (Janmey, J. Biochem. Biophys. Methods 22:41-53 (1991)), or other rheological methodologies.

"Polymerase chain reaction," or "PCR," generally refers to a method for amplification of a desired nucleotide sequence in vitro, as described, for example, in U.S. Pat. No. 4,683,195. In general, the PCR method involves repeated cycles of primer extension synthesis, using oligonucleotide primers capable of hybridizing preferentially to a template nucleic acid.

"Cell," "host cell," "cell line," and "cell culture" are used interchangeably herein and all such terms should be understood to include progeny resulting from growth or culturing of a cell. "Transformation" and "transfection" are used interchangeably to refer to the process of introducing DNA into a cell.

"Operably linked" refers to the covalent joining of two or more DNA sequences, by means of enzymatic ligation or otherwise, in a configuration relative to one another such that the normal function of the sequences can be performed. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used, in conjunction with standard recombinant DNA methods.

Amino acids are identified herein by three-letter or single-letter designations, as follows:

Asp	D	aspartic acid	Ile	I	isoleucine
Thr	T	threonine	Leu	L	leucine
Ser	S	serine	Tyr	Y	tyrosine
Glu	E	glutamic acid	Phe	F	phenylalanine
Pro	P	proline	His	H	histidine
Gly	G	glycine	Lys	K	lysine

Ala	A	alanine	Arg	R	arginine
Cys	C	cysteine	Trp	W	tryptophan
Val	V	valine	Gln	Q	glutamine
Met	M	methionine	Asn	N	asparagine

The present invention is based upon the study of structure, DNA-hydrolytic activity, and mucolytic activity of amino acid sequence variants of human DNase I. The hyperactive variants of the present invention have increased DNA-hydrolytic activity as compared to native human DNase I. The increased DNA-hydrolytic activity preferably is achieved by making mutations at and/or around those amino acid residues within native human DNase I that appear to affect the binding of DNA. Especially useful are mutations that introduce a basic amino acid residue (for example, lysine, arginine, or histidine) at one or more positions within the DNase I where the amino acid side chains are in close proximity to the negatively charged phosphate backbone of the bound DNA substrate, including, for example, at the positions of amino acid residues Gln9, Glu13, Thr14, His44, Asn74, Ser75, and Thr205 of native human DNase I (the number following the three- letter amino acid designation indicates the specific position of the amino acid residue within the Figure 1 sequence).

There are a variety of ways in which one can make hyperactive variants of human DNase I. In one embodiment of this invention, a hyperactive variant is prepared by introducing either single or multiple amino acid substitutions, insertions, and/or deletions at or adjacent to (i.e., within about five amino acid residues of) those amino acid residues of native human DNase I that affect DNA binding. Some illustrative examples of such mutations are as follows: Q9R, E13K, E13R, T14K, T14R, H44K, H44R, N74K, N74R, S75K, T205K, T205R, E13R:N74K, Q9R:E13R:N74K, E13R:N74K:T205K, Q9R:E13R:N74K:T205K (see Figures 2 and 3).

In a further embodiment of this invention, site-directed mutagenesis is used to introduce an amino acid residue at or adjacent to (i.e., within about five amino acid residues of) those amino acid residues of native human DNase I that are involved in DNA binding, which introduced residue is suitable for post-translational modification either biologically or chemically (see below). Means, et al., Chemical Modification of Proteins (Holden-Day, 1971); Glazer, et al., Chemical Modification of Proteins: Selected Methods and Analytical Procedures (Elsevier, 1975); Creighton, Proteins, pp.70-87 (W.H. Freeman, 1984); Lundblad, Chemical Reagents for Protein Modification (CRC Press, 1991). For example, a hyperactive variant of human DNase I may be produced by making post-translational modifications that increase the net positive charge at or adjacent to (i.e., within about five amino acid residues of) those amino acid residues of native human DNase I that are involved in DNA binding. For example, a cysteine residue may be introduced at or adjacent to a residue of native human DNase I that is involved in DNA binding. The free thiol of the cysteine residue then may be modified, for example, with a thiol-specific alkylating agent such as elthyleneimine which results in the formation of S-aminoethylcysteine, which carries a positive charge at neutral pH. An illustrative example of such mutations is H44C.

For convenience, substitutions, insertions, and/or deletions in the amino acid sequence of native human DNase I are usually made by introducing mutations into the corresponding nucleotide sequence of the DNA encoding native human DNase I, for example by site-directed mutagenesis. Expression of the mutated DNA then results in production of the variant human DNase I, having the desired (non-native) amino acid sequence.

Whereas any technique known in the art can be used to perform site-directed mutagenesis, e.g. as disclosed in Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor Laboratory Press, New York (1989)), oligonucleotide-directed mutagenesis is the preferred method for preparing the human DNase I variants of this invention. This method, which is well known in the art (Zoller, et al., Meth. Enz. 100:4668-500 (1983); Zoller, et al., Meth. Enz. 154:329-350 (1987); Carter, Meth. Enz. 154:382-403 (1987); Kunkel, et al., Meth. Enzymol. 154:367-382 (1987); Horwitz, et al., Meth. Enz. 185:599-611 (1990)), is particularly suitable for making substitution variants, although it may also be used to conveniently prepare deletion and insertion variants.

The site-directed mutagenesis technique typically employs a phage vector that exists in both a single-stranded and double-stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage, and plasmid vectors that contain a single-stranded phage origin of replication (Messing, et al., Meth. Enzymol. 101:20-78 (1983); Veira et al., Meth. Enzymol. 153:3-11 (1987); Short, et al., Nuc. Acids Res. 16:7583-7600 (1988)). Replication of these vectors in suitable host cells results in the synthesis of single-stranded DNA that may be used for site-directed mutagenesis.

Briefly, in carrying out site-directed mutagenesis of DNA encoding native human DNase I (or a variant thereof), the DNA is altered by first hybridizing an oligonucleotide encoding the desired mutation to a single strand of the DNA. After hybridization, a DNA polymerase is used to synthesize an entire second strand, using the hybridized oligonucleotide as a primer, and using the single strand of the DNA as a template. Thus, the oligonucleotide encoding the desired mutation is incorporated in the resulting double-stranded DNA.

Oligonucleotides for use as hybridization probes or primers may be prepared by any suitable method, such as by purification of a naturally occurring DNA or by in vitro synthesis. For example, oligonucleotides are readily synthesized using various techniques in organic chemistry, such as described by Narang, et al., Meth. Enzymol. 68:90-98 (1979); Brown, et al., Meth. Enzymol. 68:109-151 (1979); Caruthers, et al., Meth. Enzymol. 154:287-313 (1985). The general approach to selecting a suitable hybridization probe or primer is well known. Keller, et al., DNA Probes, pp. 11-18 (Stockton Press, 1989). Typically, the hybridization probe or primer will contain 10-25 or more nucleotides, and will include at least 5 nucleotides on either side of the sequence encoding the desired mutation so as to ensure that the oligonucleotide will hybridize preferentially at the desired location to the single-stranded DNA template molecule.

Of course, site-directed mutagenesis may be used to introduce multiple substitution, insertion, or deletion mutations into a starting DNA. If the sites to be mutated are located close together, the mutations may be introduced simultaneously using a single oligonucleotide that encodes all of the desired mutations. If, however, the sites to be mutated are located some distance from each other (separated by more than about ten nucleotides), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each desired mutation. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

The alternative method involves two or more rounds of mutagenesis to produce the desired variant. The first round is as described for introducing a single mutation. The second round of mutagenesis utilizes the

mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

PCR mutagenesis (Higuchi, in PCR Protocols, pp 177-183 (Academic Press, 1990); Vallette, et al., Nuc. Acids Res. 17:723-733 (1989)) is also suitable for making the variants of human DNase I. Briefly, when small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in the template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, for example, the sequence of one of the primers includes the desired mutation and is designed to hybridize to one strand of the plasmid DNA at the position of the mutation; the sequence of the other primer must be identical to a nucleotide sequence within the opposite strand of the plasmid DNA, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone. Wagner, et al., in PCR Topics, pp.69-71 (Springer-Verlag, 1991).

If the ratio of template to product amplified DNA is extremely low, the majority of product DNA fragments incorporate the desired mutation(s). This product DNA is used to replace the corresponding region in the plasmid that served as PCR template using standard recombinant DNA methods. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the plasmid fragment in a three (or more)-part ligation.

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al., Gene, 34:315-323 (1985). The starting material is the plasmid (or other vector) comprising the DNA sequence to be mutated. The codon(s) in the starting DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the DNA. The plasmid DNA is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures, wherein the two strands of the oligonucleotide are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 5' and 3' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. The resulting plasmid contains the mutated DNA sequence.

The presence of mutation(s) in a DNA is determined by methods well known in the art, including restriction mapping and/or DNA sequencing. A preferred method for DNA sequencing is the dideoxy chain termination method of Sanger, et al., Proc. Nat. Acad. Sci. USA 72:3918-3921 (1979).

DNA encoding a human DNase I variant is inserted into a replicable vector for further cloning or expression. "Vectors" are plasmids and other DNAs that are capable of replicating within a host cell, and as such, are useful for performing two functions in conjunction with compatible host cells (a vector-host system). One function is to facilitate the cloning of the nucleic acid that encodes a human DNase I variant i.e., to produce usable quantities of the nucleic acid. The other function is to direct the expression of a human DNase I variant. One or both of these functions are performed by the vector in the particular host cell used for cloning or expression. The vectors will contain different components depending upon the function they are to perform.

To produce a human DNase I variant, an expression vector will comprise DNA encoding the variant, as described above, operably linked to a promoter and a ribosome binding site. The variant then is expressed directly in recombinant cell culture, or as a fusion with a heterologous polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the junction between the heterologous polypeptide and the human DNase I variant.

Prokaryotes (e.g., E. coli, and other bacteria) are the preferred host cells for the initial cloning steps of this invention. They are particularly useful for rapid production of large amounts of DNA, for production of single-stranded DNA templates used for site-directed mutagenesis, and for DNA sequencing of the variants generated. Prokaryotic host cells also may be used for expression of DNA encoding a human DNase I variant. Polypeptides that are produced in prokaryotic cells typically will be non-glycosylated.

In addition, the human DNase I variants of this invention may be expressed in eukaryotic host cells, including eukaryotic microbes (e.g., yeast) or cells derived from an animal or other multicellular organism (e.g., Chinese hamster ovary cells, and other mammalian cells), or in live animals (e.g., cows, goats, sheep).

Cloning and expression methodologies are well known in the art. Examples of prokaryotic and eukaryotic host cells, and expression vectors, suitable for use in producing the human DNase I variants of the present invention are, for example, those disclosed in Shak, PCT Patent Publication No. WO 90/07572 (published July 12, 1990).

If prokaryotic cells or cells that contain substantial cell wall constructions are used as hosts, the preferred methods of transfection of the cells with DNA is the calcium treatment method described by Cohen et al., Proc. Natl. Acad. Sci. 69:2110-2114 (1972) or the polyethylene glycol method of Chung et al., Nuc. Acids. Res. 16:3580 (1988). If yeast are used as the host, transfection is generally accomplished using polyethylene glycol, as taught by Hinnen, Proc. Natl. Acad. Sci. U.S.A., 75: 1929-1933 (1978). If mammalian cells are used as host cells, transfection generally is carried out by the calcium phosphate precipitation method, Graham, et al., Virology 52:546 (1978), Gorman, et al., DNA and Protein Eng. Tech. 2:3-10 (1990). However, other known methods for introducing DNA into prokaryotic and eukaryotic cells, such as nuclear injection, electroporation, or protoplast fusion also are suitable for use in this invention.

Particularly useful in this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding human DNase I variants. In general, transient expression involves the use of an expression vector that is able to efficiently replicate in a host cell, such that the host cell accumulates many

copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Wong, et al., Science 228:810-815 (1985); Lee, et al., Proc. Nat Acad. Sci. USA 82:4360-4364 (1985); Yang, et al., Cell 47:3-10 (1986). Thus, transient expression systems are conveniently used for expressing the DNA encoding amino acid sequence variants of native human DNase I, in conjunction with assays to identify those variants that have increased DNA-hydrolytic activity.

A human DNase I variant preferably is secreted from the host cell in which it is expressed, in which case the variant is recovered from the culture medium in which the host cells are grown. In that case, it may be desirable to grow the cells in a serum free culture medium, since the absence of serum proteins and other serum components in the medium may facilitate purification of the variant. If it is not secreted, then the human DNase I variant is recovered from lysates of the host cells. When the variant is expressed in a host cell other than one of human origin, the variant will be completely free of proteins of human origin. In any event, it will be necessary to purify the variant from recombinant cell proteins in order to obtain substantially homogeneous preparations of the human DNase I variant. For therapeutic uses, the purified variant preferably will be greater than 99% pure (i.e., any other proteins will comprise less than 1% of the total protein in the purified composition).

Generally, purification of a human DNase I variant is accomplished by taking advantage of the differential physicochemical properties of the variant as compared to the contaminants with which it may be associated. For example, as a first step, the culture medium or host cell lysate is centrifuged to remove particulate cell debris. The human DNase I variant thereafter is purified from contaminant soluble proteins and polypeptides, for example, by ammonium sulfate or ethanol precipitation, gel filtration (molecular exclusion chromatography), ion-exchange chromatography, hydrophobic chromatography, immunoaffinity chromatography (e.g., using a column comprising anti-human DNase I antibodies coupled to Sepharose) tentacle cation exchange chromatography (Frenz, et al., PCT Patent Publication No. WO 93/25670, published December 23, 1993), reverse phase HPLC, and/or gel electrophoresis.

Of course, one skilled in the art will appreciate that the purification methods that are used for native human DNase I may require some modification to be useful in purifying a human DNase I variant, to account for structural and other differences between the native and variant proteins. For example, in some host cells (especially bacterial host cells) the human DNase I variant may be expressed initially in an insoluble, aggregated form (referred to in the art as "refractile bodies" or "inclusion bodies") in which case it will be necessary to solubilize and renature the human DNase I variant in the course of its purification. Methods for solubilizing and renaturing recombinant protein refractile bodies are known in the art (see e.g., Builder, et al., U.S. Patent No. 4,511,502).

In another embodiment of this invention, covalent modifications are made to a native or variant human DNase I protein to increase the DNA-hydrolytic activity of the protein or to affect another property of the protein (e.g., stability, biological half-life, immunogenicity). Such covalent modifications may be made instead of or in addition to the amino acid sequence substitution, insertion, and deletion mutations described above.

Covalent modifications may be introduced by reacting targeted amino acid residues of the native or variant human DNase I with an organic derivatizing agent that is capable of reacting with selected amino acid side-chains or N- or C-terminal residues. Suitable derivatizing agents and methods are well known in the art.

For example, cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate,2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'-N=C=N-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Covalent coupling of glycosides to amino acid residues of a native or variant human DNase I protein may be used to modify or increase the number or profile of carbohydrate substituents, especially at or adjacent to those residues that are involved in DNA binding. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan or (f) the amide group of glutamine. Suitable methods are described, for example in PCT Patent Publication No. WO 87/05330 (published September 11, 1987), and in Aplin, et al., CRC Crit. Rev. Biochem., pp. 259-306 (1981).

The covalent attachment of agents such as polyethylene glycol (PEG) or human serum albumin to human DNase I variants may reduce immunogenicity and/or toxicity of the variant and/or prolong its half-life, as has been observed with other proteins. Abuchowski, et al., J. Biol. Chem. 252:3582-3586 (1977); Poznansky, et al., FEBS Letters 239:18-22 (1988); Goodson, et al., Biotechnology 8:343-346 (1990); Katre, J. Immunol. 144:209-213 (1990); Harris, Polyethylene Glycol Chemistry (Plenum Press, 1992).

In a further embodiment of this invention, a hyperactive variant of human DNase I will comprise one or more additional amino acid sequence mutations or other covalent modifications that causes the variant to have reduced binding affinity for actin. Examples of such mutations and covalent modifications that reduce actin binding are described in PCT Patent Publication WO 96/26279. A hyperactive variant also may comprise an amino acid sequence mutation or other covalent modification that reduces the susceptibility of the variant to degradation by proteases (e.g., neutrophil elastase) that may be present in sputum and other biological materials.

The DNA-hydrolytic activity of the human DNase I variants prepared as described above are readily determined using assays and methods known in the art and as described herein. Any such variant having increased DNA-hydrolytic activity (as defined herein) is a hyperactive variant within the scope of this invention.

Antibodies to hyperactive variants of human DNase I are produced by immunizing an animal with a hyperactive variant or a fragment thereof, optionally in conjunction with an immunogenic polypeptide, and thereafter recovering antibodies from the serum of the immunized animals. Alternatively, monoclonal antibodies are prepared from cells of the immunized animal in conventional fashion. The antibodies also can be made in the form of chimeric (e.g., humanized) or single chain antibodies or Fab fragments, using methods well known in the art. Preferably, the antibodies will bind to the hyperactive variant but will not substantially bind to (i.e., cross react with) other DNase proteins (such as native human and bovine DNase I). The antibodies can be used in methods relating to the localization and activity of the hyperactive variant, for example, for detecting and measuring its levels in tissues or clinical samples. Immobilized antibodies are particularly useful in the detection of the hyperactive variant in clinical samples for diagnostic purposes, and in the purification of the hyperactive variant, for example from recombinant cell cultures.

The hyperactive variants of human DNase I that are provided by this invention are used to reduce the viscoelasticity of DNA-containing material, including sputum, mucus, or other pulmonary secretions. Such variants are particularly useful for the treatment of patients with pulmonary disease who have abnormal viscous or inspissated secretions and conditions such as acute or chronic bronchial pulmonary disease, including infectious pneumonia, bronchitis or tracheobronchitis, bronchiectasis, cystic fibrosis, asthma, tuberculosis, and fungal infections. For such therapies, a solution or finely divided dry preparation of the hyperactive variant is instilled in conventional fashion into the airways (e.g., bronchi) or lungs of a patient, for example by aerosolization.

The hyperactive variants are also useful for adjunctive treatment of abscesses or severe closed-space infections in conditions such as empyema, meningitis, abscess, peritonitis, sinusitis, otitis, periodontitis, pericarditis, pancreatitis, cholelithiasis, endocarditis and septic arthritis, as well as in topical treatments in a variety of inflammatory and infected lesions such as infected lesions of the skin and/or mucosal membranes, surgical wounds, ulcerative lesions and burns. The hyperactive variant may improve the efficacy of antibiotics used in the treatment of such infections (e.g., gentamicin activity is markedly reduced by reversible binding to intact DNA).

Hyperactive variants of human DNase I will be useful for the treatment of systemic lupus erythematosus (SLE), a life-threatening autoimmune disease characterized by the production of diverse autoantibodies. DNA is a major antigenic component of the immune complexes. In this instance, the

hyperactive human DNase I (native or variant) may be given systemically, for example by intravenous, subcutaneous, intrathecal, or intramuscular administration to the affected patient.

Hyperactive variants of human DNase I also will be useful for preventing the new development and/or exacerbation of respiratory infections, such as may occur in patients having cystic fibrosis, chronic bronchitis, asthma, pneumonia, or other pulmonary disease, or patients whose breathing is assisted by ventilator or other mechanical device, or other patients at risk of developing respiratory infections, for example post-surgical patients.

The hyperactive variants of the invention can be formulated according to known methods to prepare therapeutically useful compositions. A preferred therapeutic composition is a solution of a hyperactive variant in a buffered or unbuffered aqueous solution, and preferably is an isotonic salt solution such as 150 mM sodium chloride containing 1.0 mM calcium chloride at pH 7. These solutions are particularly adaptable for use in commercially-available nebulizers including jet nebulizers and ultrasonic nebulizers useful for administration directly into the airways or lungs of an affected patient.

In another embodiment, the therapeutic composition comprises a dry powder of the hyperactive variant, preferably prepared by spray-drying of a solution of the variant, essentially as described in PCT Patent Publication WO 95/23613.

In a further embodiment, the therapeutic composition comprises cells actively producing a hyperactive variant of human DNase I. Such cells may be directly introduced into the tissue of a patient, or may be encapsulated within porous membranes which are then implanted in a patient, in either case providing for the delivery of the hyperactive variant into areas within the body of the patient in need of increased DNA-hydrolytic activity. For example, the patient's own cells could be transformed, either in vivo or ex vivo, with DNA encoding a hyperactive variant of human DNase I, and then used to produce the variant DNase I directly within the patient. This latter method is commonly referred to as gene therapy.

The therapeutically effective amount of a hyperactive variant of human DNase I will depend, for example, upon the amount of DNA in the material to be treated, the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. In view of its increased DNA-hydrolytic activity, the amount of the hyperactive variant required to achieve a therapeutic effect may be less than the amount of native human DNase I necessary to achieve the same effect under the same conditions. Generally, the therapeutically effective amount of the hyperactive variant will be a dosage of from about 0.1 μg to about 5 mg of the variant per kilogram of body weight of the patient, administered within pharmaceutical compositions, as described herein.

A hyperactive human DNase I variant optionally is combined with or administered in concert with one or more other pharmacologic agents used to treat the conditions listed above, such as antibiotics, bronchodilators, anti-inflammatory agents, mucolytics (e.g. n-acetyl-cysteine), actin binding or actin severing proteins (e.g., gelsolin; Matsudaira et al., Cell 54:139-140 (1988); Stossel, et al., PCT Patent Publication No. WO 94/22465 (published October 13, 1994)), protease inhibitors, gene therapy product (e.g., comprising the cystic fibrosis transmembrane conductance regulator (CFTR) gene, Riordan, et al., Science 245:1066-1073 (1989)), glucocorticoids, or cytotoxic agents.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated.

EXAMPLE 1

Mutagenesis of Human DNase I

E. coli strain CJ236 (BioRad Laboratories, Richmond, California USA) was transformed with plasmid pRK.DNase.3 using the method of Chung et al. (Nuc. Acids. Res. 16:3580 (1988). The plasmid pRK.DNase.3 used in making the present invention is as described in PCT Patent Publication No. WO 90/07572 (published July 12, 1990), except that the nucleotide sequence encoding the mature human DNase I is as shown in Figure 1A of Shak, et al., Proc. Nat. Acad. Sci. 87:9188-9192 (1990). Transformed cells were plated on LB agar plates containing 50 μg/ml carbenicillin and grown overnight at 37°C. 2YT broth (5 ml) containing 50 μg/ml carbenicillin and 10 μl VCSM13 helper phage (Stratagene, La Jolla, California USA) was inoculated with an individual colony from the agar plate and grown overnight at 37°C with agitation. Single stranded DNA was isolated from this culture and used as template for subsequent mutagenesis.

Site-directed mutagenesis was accomplished using synthetic oligonucleotides according to the method of Kunkel, et al. (Meth. Enzymol. 154: 367-382 (1987). The mutagenic oligonucleotides were 27-mers having 12 exact base matches 5' to the mismatched codon and 12 exact base matches 3' to the mismatched codon. Following mutagenesis, single stranded DNA from individual clones was subjected to dideoxy sequencing (Sanger, et al., Proc. Nat. Acad. Sci. USA 74: 5463-5467 (1977)). DNA having variant nucleotide sequences then was transformed as described above into E. coli strain XL1 Blue MRF' (Stratagene). After plating and single colony isolation as before, individual colonies were used to inoculate 0.5 liter LB broth containing 50 ug/ml carbenicillin. Following growth overnight with agitation at 37°C, the cells were harvested by centrifugation and the variant DNA (in the expression vector) was purified using Qiagen tip-500 columns (Qiagen Inc., Chatsworth, California USA).

Figures 2 and 3 identify the different human DNase I variants that were made. In the figures and throughout the specification, the description of the amino acid substitution mutation(s) present in a DNase I variant is abbreviated by a first alphabetical letter, a number, and a second alphabetical letter. The first alphabetical letter is the single letter abbreviation of amino acid residue in native (wild-type) human mature DNase I, the number indicates the position of that residue in native human mature DNase I (numbering as shown in Figure 1), and the second alphabetical letter is the single letter abbreviation of the amino acid residue at that position in the variant DNase I. For example, in the DNase I variant having a E13R mutation, the glutamic acid (E) residue at position 13 in native human mature DNase I has been replaced by an arginine (R) residue. Multiple mutations in a single variant are designated similarly, with a colon (:) separating each of the different mutations that are present in the variant. For example, the designation E13R:N74K indicates that the variant has a E13R mutation and a N74K mutation.

EXAMPLE 2

Expression of Human DNase I Variants

Human embryonic kidney 293 cells (ATCC CRL 1573, American Type Culture Collection, Rockville, Maryland USA) were grown in serum containing media in 150 mm plastic Petri dishes. Log phase cells were transiently cotransfected with 22.5 μg purified variant DNA (prepared as described above) and 17 μg adenovirus DNA using the calcium phosphate precipitation method (Gorman, et al., DNA and Protein Eng. Tech. 2:3-10 (1990)). Approximately 16 hours after transfection, the cells were washed with 15 ml phosphate buffered saline and the media was changed to serum free media. Cell culture media was harvested from each plate at about 96 hours following the serum free media change. A total of approximately 25 ml of cell culture supernatant containing the DNase I variant was obtained in this way. The pool of culture supernatant from each plate was concentrated about 10-fold using Centriprep 10 concentrators. The concentration of DNase I protein in the concentrates was determined using a DNase I protein ELISA as described in PCT Patent Publication WO 96/26279.

Culture supernatant containing native human DNase I was prepared by the same procedure as described above, except that the 293 cells were transiently transfected with plasmid pRK.DNase.3.

EXAMPLE 3

Biochemical Activities of Human DNase I Variants

I. Plasmid DNA Digestion Assays

To determine the DNA-hydrolytic activity of human DNase I variants, two different plasmid digestion assays were used. The first assay ("supercoiled DNA digestion assay") measured the conversion of supercoiled double-stranded pBR322 plasmid DNA to relaxed (nicked), linear, and degraded forms. The second assay ("linear DNA digestion assay") measured the conversion of linear double-stranded pBR322 DNA to degraded forms.

Specifically, culture supernatants (prepared as described above, and diluted approximately 1:1000 before use) were added to 160 μl of solution containing 25 μg/ml of either supercoiled pBR322 DNA or EcoRI-digested linearized pBR322 DNA in 25 mM HEPES, pH 7.1, 100 μg/ml bovine serum albumin, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 150 mM NaCl, and the samples were incubated at room temperature. At various times, aliquots of the reaction mixtures were removed and quenched by the addition of 25 mM EDTA, together with xylene cyanol, bromphenol blue, and glycerol. The integrity of the pBR322 DNA in the quenched samples was analyzed by electrophoresis of the samples on 0.8% weight/vol. agarose gels. After electrophoresis, the gels were stained with a solution of ethidium bromide and the DNA in the gels was visualized by ultraviolet light. The relative amounts of supercoiled, relaxed, and linear forms of pBR322 DNA were determined by scanning of the gels with a Molecular Dynamics Model 575 FluorImager and quantitating the amount of DNA in the bands of the gel that corresponded to those different forms.

The results of these assays are shown in Figure 2. In the supercoiled DNA digestion assay, the overall activity of the human DNase I variants was measured as the initial rate of disappearance of supercoiled DNA

(as a result of it being converted to relaxed (nicked), linear, or degraded DNA), normalized relative to the rate observed with native human DNase I ("relative nicking activity"). The ratio of linearized to relaxed forms of pBR322 DNA also was determined relative to that observed with native human DNase I ("L/R ratio"). In the linear DNA digestion assay, the activity of the human DNase I variants was measured as the initial rate of disappearance of linear DNA (as a result of it being converted to degraded forms), normalized relative to the rate observed with native human DNase I ("relative linear DNA digestion activity"). In the supercoiled DNA digestion assay, native human DNase I had a supercoiled DNA nicking activity of 1200 ± 43 mg DNA min^{-1} mg^{-1} DNase I (n=2), and gave a linear to relaxed product ratio of 0.010. In the linear DNA digestion assay, native human DNase I had a linear DNA digestion activity of 23 ± 3 mg DNA min^{-1} mg^{-1} DNase I (n=6).

II. Hyperchromicity Assay

The DNA-hydrolytic activity of human DNase I variants also was determined using a hyperchromicity assay which is based on the increase in absorbance at 260 nm upon denaturation and depolymerization of DNA (Kunitz, J. Gen. Physiol. 33:349-362 (1950); Kunitz, J. Gen. Physiol. 33:363-377 (1950)).

In the hyperchromicity assay, culture supernatants (prepared as described above, and diluted approximately 1:2 to 1:50 before use) were added to 150 µl of solution containing 10 µg/ml to 600 µg/ml calf thymus DNA in 25 mM HEPES, pH 7.1, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 150 mM NaCl, and the increase in absorbance at 260 nm was monitored with a spectrophotometer (Molecular Devices Spectra Max 250) for six minutes. Plots of activity versus DNA concentration were hyperbolic and the data were fit to the Michaelis-Menton equation to generate K_m and V_{max} kinetic values. Figure 3 shows $1/K_m$, V_{max}, and V_{max}/K_m values calculated for the human DNase I variants which are normalized relative to those of native human DNase I. In this assay, native human DNase I had a K_m of 229 ± 33 µg/ml DNA (n=6) and a V_{max} of 168 ± 18 A_{260} units min^{-1} mg^{-1} DNase I (n=6).

III. Effect of Sodium Chloride on DNA-Hydrolytic Activity

The effect of sodium chloride on DNA-hydrolytic activity of several human DNase I variants was determined using the linear DNA digestion assay essentially as described above, except that sodium chloride was added to the reaction mixtures to a final concentration of 20 mM to 400 mM. Figure 4 shows the percent activity of hyperactive variants and native human DNase I at various concentrations of sodium chloride, relative to their respective activities in the absence of added sodium chloride.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Genentech, Inc.

(ii) TITLE OF INVENTION: HUMAN DNASE I HYPERACTIVE VARIANTS

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
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(B) FILING DATE 09-JUN-1997
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: 08/663831
(B) FILING DATE: 14-JUN-1996

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
 1               5                  10                  15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                20                  25                  30
```

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu Lys Ile Ala Ala Phe Asn Ile Arg Thr Phe Gly Glu Thr Lys

1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                20                  25                  30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
                35                  40                  45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
                50                  55                  60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
                65                  70                  75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
                80                  85                  90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
                95                 100                 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
               110                 115                 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
               125                 130                 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
               140                 145                 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
               155                 160                 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
               170                 175                 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
               185                 190                 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
               200                 205                 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
               215                 220                 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
               230                 235                 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
               245                 250                 255

Glu Val Met Leu Lys
               260
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Arg Thr Lys
 1               5                  10                  15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                20                  25                  30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
                35                  40                  45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
                50                  55                  60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
                65                  70                  75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
                80                  85                  90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
                95                 100                 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
               110                 115                 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
               125                 130                 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
               140                 145                 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
               155                 160                 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
               170                 175                 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
               185                 190                 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
               200                 205                 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
               215                 220                 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
               230                 235                 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
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245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Lys Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Arg Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala

200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser Lys Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser Arg Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu

155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Lys Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

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Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
                140                     145                 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
                155                     160                 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
                170                     175                 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
                185                     190                 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
                200                     205                 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
                215                     220                 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
                230                     235                 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
                245                     250                 255

Glu Val Met Leu Lys
                260
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
  1             5                       10                  15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                20                      25                  30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
                35                      40                  45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
                50                      55                  60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Arg Ser
                65                      70                  75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
                80                      85                  90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
                95                      100                 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
```

110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Lys
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser

```
                        65                        70                        75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
                80                  85                  90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
                95                  100                 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
                110                 115                 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
                125                 130                 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
                140                 145                 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
                155                 160                 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
                170                 175                 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
                185                 190                 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Lys Pro Thr His Cys Ala
                200                 205                 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
                215                 220                 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
                230                 235                 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
                245                 250                 255

Glu Val Met Leu Lys
                260
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Glu Thr Lys
1               5                   10                  15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                20                  25                  30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
                35                  40                  45
```

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Asn Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Arg Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Arg Thr Lys
1 5 10 15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser

20 25 30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
35 40 45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
50 55 60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Lys Ser
65 70 75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
80 85 90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
95 100 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
110 115 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
125 130 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
140 145 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
155 160 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
170 175 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
185 190 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
200 205 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
215 220 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
230 235 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
245 250 255

Glu Val Met Leu Lys
260

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Leu Lys Ile Ala Ala Phe Asn Ile Arg Thr Phe Gly Arg Thr Lys
 1               5                  10                  15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                20                  25                  30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
                35                  40                  45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
                50                  55                  60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Lys Ser
                65                  70                  75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
                80                  85                  90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
                95                 100                 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
               110                 115                 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
               125                 130                 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
               140                 145                 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
               155                 160                 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
               170                 175                 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
               185                 190                 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Thr Pro Thr His Cys Ala
               200                 205                 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
               215                 220                 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
               230                 235                 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
               245                 250                 255

Glu Val Met Leu Lys
               260
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids

(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Leu Lys Ile Ala Ala Phe Asn Ile Gln Thr Phe Gly Arg Thr Lys
1               5                   10                  15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                20                  25                  30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
                35                  40                  45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
                50                  55                  60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Lys Ser
                65                  70                  75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
                80                  85                  90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
                95                  100                 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
                110                 115                 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
                125                 130                 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
                140                 145                 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
                155                 160                 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
                170                 175                 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
                185                 190                 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Lys Pro Thr His Cys Ala
                200                 205                 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
                215                 220                 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
                230                 235                 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
                245                 250                 255

Glu Val Met Leu Lys
                260
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Leu Lys Ile Ala Ala Phe Asn Ile Arg Thr Phe Gly Arg Thr Lys
  1               5                  10                  15

Met Ser Asn Ala Thr Leu Val Ser Tyr Ile Val Gln Ile Leu Ser
                 20                  25                  30

Arg Tyr Asp Ile Ala Leu Val Gln Glu Val Arg Asp Ser His Leu
                 35                  40                  45

Thr Ala Val Gly Lys Leu Leu Asp Asn Leu Asn Gln Asp Ala Pro
                 50                  55                  60

Asp Thr Tyr His Tyr Val Val Ser Glu Pro Leu Gly Arg Lys Ser
                 65                  70                  75

Tyr Lys Glu Arg Tyr Leu Phe Val Tyr Arg Pro Asp Gln Val Ser
                 80                  85                  90

Ala Val Asp Ser Tyr Tyr Tyr Asp Asp Gly Cys Glu Pro Cys Gly
                 95                 100                 105

Asn Asp Thr Phe Asn Arg Glu Pro Ala Ile Val Arg Phe Phe Ser
                110                 115                 120

Arg Phe Thr Glu Val Arg Glu Phe Ala Ile Val Pro Leu His Ala
                125                 130                 135

Ala Pro Gly Asp Ala Val Ala Glu Ile Asp Ala Leu Tyr Asp Val
                140                 145                 150

Tyr Leu Asp Val Gln Glu Lys Trp Gly Leu Glu Asp Val Met Leu
                155                 160                 165

Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Arg Pro Ser Gln
                170                 175                 180

Trp Ser Ser Ile Arg Leu Trp Thr Ser Pro Thr Phe Gln Trp Leu
                185                 190                 195

Ile Pro Asp Ser Ala Asp Thr Thr Ala Lys Pro Thr His Cys Ala
                200                 205                 210

Tyr Asp Arg Ile Val Val Ala Gly Met Leu Leu Arg Gly Ala Val
                215                 220                 225

Val Pro Asp Ser Ala Leu Pro Phe Asn Phe Gln Ala Ala Tyr Gly
                230                 235                 240

Leu Ser Asp Gln Leu Ala Gln Ala Ile Ser Asp His Tyr Pro Val
```

245 250 255

Glu Val Met Leu Lys
260

Claims

What is claimed is:

1. A human DNase I hyperactive variant.

2. A variant of claim 1 that has DNA-hydrolytic activity that is at least 50% greater than that of native human DNase I as determined in a linear DNA digestion assay.

3. A variant of claim 1 that has DNA-hydrolytic activity that is at least 2-fold greater than that of native human DNase I as determined in a linear DNA digestion assay.

4. A variant of claim 1 comprising an amino acid sequence having at least 90% identity with the amino acid sequence of native human DNase I shown in Figure 1.

5. A variant of claim 1 comprising an amino acid sequence having at least 95% identity with the amino acid sequence of native human DNase I shown in Figure 1.

6. A human DNase I hyperactive variant having an amino acid sequence that differs from the amino acid sequence shown in Figure 1 by the substitution of one amino acid for another at only a single position within the Figure 1 sequence.

7. A variant of claim 6 wherein the amino acid substitution is at one of the following positions within the Figure 1 sequence: Gln9, Glu13, Thr14, His44, Asn74, Ser75, and Thr205.

8. A human DNase I hyperactive variant having an amino acid sequence that differs from the amino acid sequence shown in Figure 1 by the substitution of one amino acid for another at two or more positions within the Figure 1 sequence.

9. A variant of claim 8 wherein at least one of the amino acid substitutions is made at one of the following positions within the Figure 1 sequence: Gln9, Glu13, Thr14, His44, Asn74, Ser75, and Thr205.

10. An isolated nucleic acid encoding a human DNase I hyperactive variant.

11. The nucleic acid of claim 10 comprising a nucleotide sequence that encodes an amino acid sequence having at least 90% identity with the amino acid sequence of native human DNase shown in Figure 1.

12. The nucleic acid of claim 10 comprising a nucleotide sequence that encodes an amino acid sequence having at least 95% identity with the amino acid sequence of native human DNase shown in Figure 1.

13. The nucleic acid of claim 10 comprising a nucleotide sequence that encodes an amino acid sequence that differs from the amino acid sequence shown in Figure 1 by the substitution of one amino acid for another at only a single position within the Figure 1 sequence.

14. The nucleic acid of claim 10 comprising a nucleotide sequence that encodes an amino acid sequence that differs from the amino acid sequence shown in Figure 1 by the substitution of one amino acid for another at two or more positions within the Figure 1 sequence.

15. A method for the treatment of a patient having a pulmonary disease or disorder comprising administering to the patient a therapeutically effective amount of a human DNase I hyperactive variant.

16. The method of claim 15 wherein the disease or disorder is cystic fibrosis.

17. A method for the treatment of a patient having systemic lupus erythematosus comprising administering to the patient a therapeutically effective amount of a human DNase I hyperactive variant.

18. A pharmaceutical composition comprising a human DNase I hyperactive variant and optionally a pharmaceutically acceptable excipient.

19. The composition of claim 18 wherein the composition is in liquid form.

20. The composition of claim 18 wherein the composition is in powder form.

Figure 1

Human Mature DNase I

```
         10        20        30        40        50
LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALVQEVRDSHLTAVGK

         60        70        80        90       100
LLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDG

        110       120       130       140       150
CEPCGNDTFNREPAIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALYDV

        160       170       180       190       200
YLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSA

        210       220       230       240       250
DTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAYGLSDQLAQAIS

        260
DHYPVEVMLK
```

Figure 2

Plasmid DNA Digestion Assays

DNase I Variants	Linear DNA Digestion Assay: Relative Linear DNA Digestion Activity	Supercoiled DNA Digestion Assay: L/R ratio	Supercoiled DNA Digestion Assay: Relative Nicking Activity
native human DNase I	1.0 ± 0.1	1.0	1.0 ± 0.0
Q9R	3.5 ± 0.4	2.3	3.4 ± 0.5
E13K	3.9 ± 0.1		
E13R	6.0 ± 0.5	5.4	2.2 ± 0.0
T14K	4.2 ± 0.1	4.7	2.9 ± 0.8
T14R	3.5 ± 0.7		
H44K	2.0 ± 0.4	2.3	1.8 ± 0.3
H44R	3.6 ± 0.5		
N74K	6.0 ± 0.1	4.7	7.3 ± 1.0
N74R	4.1 ± 0.8		
S75K	1.5 ± 0.2		
T205K	4.7 ± 0.2	5.4	2.8 ± 0.7
T205R	2.3 ± 0.3		
E13R:N74K	26.7 ± 4.1	12.3	6.9 ± 1.6
Q9R:E13R:N74K	38.3 ± 1.2	16.5	6.3 ± 2.2
E13R:N74K:T205K	19.5 ± 6.4		
Q9R:E13R:N74K:T205K	30.5 ± 7.5		

All data is normalized to native human DNase I.

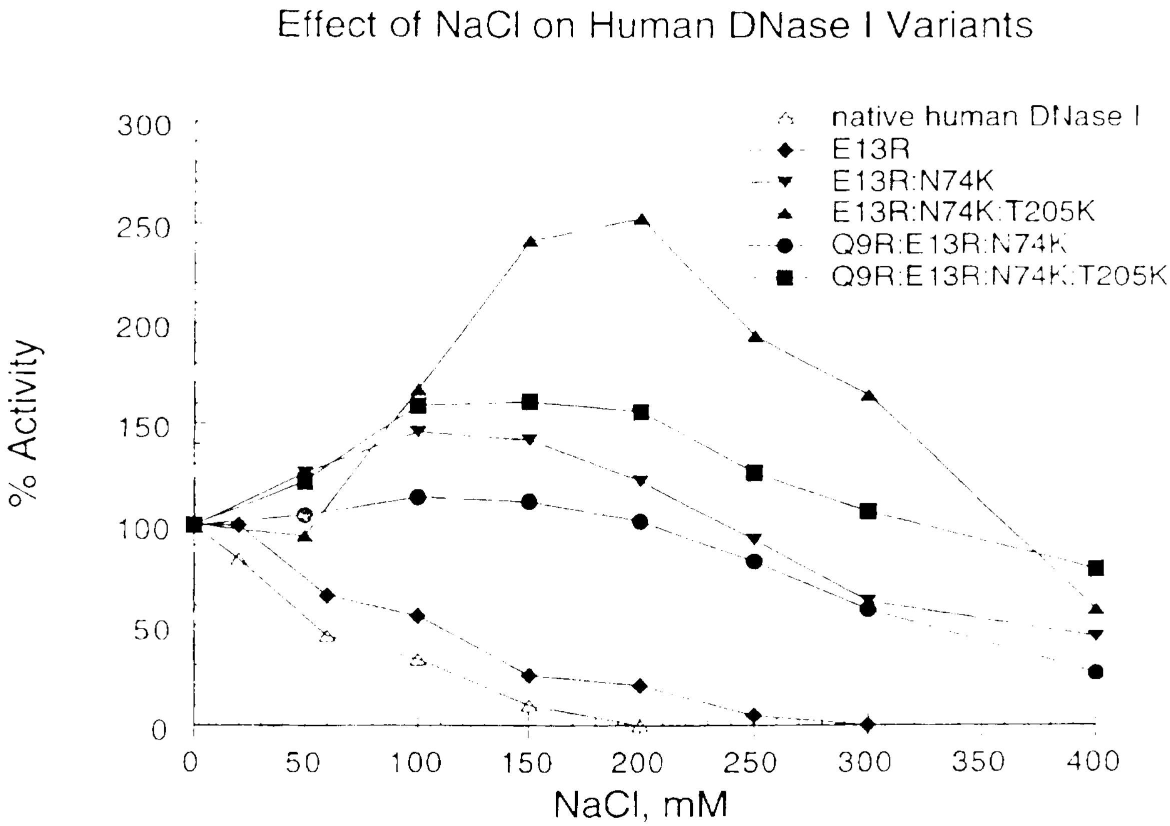
Figure 3

DNA Hyperchromicity Assay

DNase I Variants	$1/K_m$	V_{max}	V_{max}/K_m
native human DNase I	1.0 ± 0.1	1.0 ± 0.1	1.0
Q9R	0.9 ± 0.2	2.8 ± 0.4	2.6
E13K	2.5 ± 0.4	1.8 ± 0.1	4.5
E13R	4.3 ± 1.4	1.5 ± 0.1	6.5
T14K	2.3 ± 0.9	1.1 ± 0.2	2.5
T14R	2.1 ± 0.8	0.7 ± 0.1	1.5
H44K	2.3 ± 0.5	1.1 ± 0.1	2.5
H44R	1.7 ± 0.2	1.0 ± 0.1	1.7
N74K	0.4 ± 0.2	5.5 ± 1.3	2.3
N74R	2.6 ± 0.8	3.1 ± 0.3	8.1
S75K	18.5 ± 2.0	0.4 ± 0.1	7.4
T205K	2.4 ± 0.8	2.1 ± 0.4	5.0
T205R	3.0 ± 1.2	1.0 ± 0.1	3.0
E13R:N74K	5.0 ± 1.7	5.3 ± 0.5	26.5
Q9R:E13R:N74K	4.9 ± 1.3	7.0 ± 0.4	34.3
E13R:N74K:T205K	5.0 ± 1.9	6.3 ± 0.6	31.5
Q9R:E13R:N74K:T205K	5.6 ± 1.4	3.8 ± 0.3	21.3

All data is normalized to native human DNase I.

Figure 4


Effect of NaCl on Human DNase I Variants
native human DNase I
E13R
E13R:N74K
E13R:N74K:T205K
Q9R:E13R:N74K
Q9R:E13R:N74K:T205K
% Activity
0
50
100
150
200
250
300
0
50
100
150
200
250
300
350
400
NaCl, mM

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 97/08517

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/55 C12N9/22 A61K38/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	SUCK D: "DNA recognition by DNase I." J MOL RECOGNIT, JUN 1994, 7 (2) P65-70, ENGLAND, XP002040573 see the whole document ---	1-14
A	LIAO, TA HSIU ET AL: "Bovine pancreatic deoxyribonuclease A. Isolation of cyanogen bromide peptides, complete covalent structure of the polypeptide chain. [Erratum to document cited in CA78(19):120847p]" J. BIOL. CHEM. (1992), 267(11), 7957 CODEN: JBCHA3;ISSN: 0021-9258, XP002040574 see the whole document ---	1-14

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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1997	28.10.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WORRALL AF ET AL: "The chemical synthesis of a gene coding for bovine pancreatic DNase I and its cloning and expression in Escherichia coli." J BIOL CHEM, DEC 15 1990, 265 (35) P21889-95, UNITED STATES, XP002040575 see the whole document ---	1-14
A	WO 90 07572 A (GENENTECH INC) 12 July 1990 see the whole document ---	1-14
A	WO 93 25670 A (GENENTECH INC) 23 December 1993 see claims 1-19 ---	1-14
P,X	WO 96 26278 A (GENENTECH INC ;LAZARUS ROBERT A (US); SHAK STEVEN (US); ULMER JANA) 29 August 1996 see figures 2,5 ---	1,6,7
P,X	WO 96 26279 A (GENENTECH INC) 29 August 1996 see figure 5A -----	1,6,7

2

INTERNATIONAL SEARCH REPORT	International application No PCT/US 97/08517

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1 [X] Claims Nos
because they relate to subject matter not required to be searched by this Authority, namely
Remark: Although claim(s) 15-17
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2 [ ] Claims Nos
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically

3 [ ] Claims Nos
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows

1 [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims

2 [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee

3 [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos

4 [ ] No required additional search fees were timely paid by the applicant Consequently, this International Search Report is restricted to the invention first mentioned in the claims, it is covered by claims Nos

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest

[ ] No protest accompanied the payment of additional search fees

INTERNATIONAL SEARCH REPORT

I...rmation on patent family members

Interna' al Application No
PCT/US 97/08517

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9007572 A	12-07-90	AU 630658 B	05-11-92
		AU 4826590 A	01-08-90
		CA 2006473 A	23-06-90
		EP 0449968 A	09-10-91
		HU 211232 B	28-11-95
		JP 4502406 T	07-05-92
WO 9325670 A	23-12-93	US 5279823 A	18-01-94
		AU 4398193 A	04-01-94
		BG 99234 A	28-07-95
		CA 2137237 A	23-12-93
		CZ 9403032 A	14-06-95
		DE 4392749 T	20-07-95
		EP 0644932 A	29-03-95
		FI 945549 A	25-11-94
		GB 2282140 A,B	29-03-95
		HU 70468 A	30-10-95
		IL 105724 A	10-06-97
		JP 7507455 T	24-08-95
		NO 944752 A	08-12-94
		NZ 253559 A	26-11-96
		SK 149594 A	10-01-96
WO 9626278 A	29-08-96	AU 1970395 A	11-09-96
		AU 5026396 A	11-09-96
		WO 9626279 A	29-08-96
WO 9626279 A	29-08-96	WO 9626278 A	29-08-96
		AU 1970395 A	11-09-96
		AU 5026396 A	11-09-96